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Evaluation of the Tentative Standard Method for Enteric Virus Concentration from Large Volumes of Tap Water

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16. ABSTRACT

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Evaluation of the Tentative Standard Method for Enteric Virus Concentration From Large Volumes of Tap Water

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During 24 months of evaluation the tentative standard method was found to be inefficient—and sometimes ineffective—for concentrating low levels of laboratory-grown and naturally occurring sewage-extracted enteric viruses from 380-L (100-gal) volumes of experimentally contaminated tap water.

The continued occurrence of waterborne outbreaks of hepatitis A (infectious hepatitis) and perhaps other viral diseases such as viral gastroenteritis,[1,2] as well as the apparent isolation of enteric viruses from drinking water supplies in the US[3,4] and elsewhere,[5-7] indicate the need for a more thorough and systematic evaluation of the public health problem of virus transmission via drinking water.[8-10] Evaluation of this problem requires the development and utilization of simple and reliable methods to quantitatively detect relatively small numbers of enteric viruses in large volumes of raw and finishing drinking water supplies.[11,12] Information on virus levels in drinking water supplies, along with epidemiological information on waterborne viral infection and illness, may make it possible to assess the risk to the public of waterborne viral disease transmission.

Research efforts on methods to detect enteric viruses in water have produced two-step microporous filter adsorption-elution methods that are sometimes capable of concentrating small numbers of enteroviruses from 380 L (100 gal) or more of drinking water,[11-16] and a tentative method of this type was introduced in the 14th edition of *Standard Methods*.[17]

The development of flow-through filter adsorption-elution systems for concentrating small quantities of viruses from large volumes of water represents a much-needed technical achievement. However, many of the key variables involved in this type of methodology have not been adequately evaluated, and some of the procedures and equipment in current use have been somewhat arbitrarily selected. A rational and systematic evaluation of the methodology and materials is needed.

In particular, it has not been demonstrated that the tentative standard method (TSM) is capable of efficiently detecting viruses of all four major enteric virus groups. The method was developed and evaluated primarily for enteroviruses, especially poliovirus, and information in the literature indicates that the method may be incapable of recovering some other enteric viruses. For example, in the application of filter adsorption-elution methods to adenoviruses, Fields[18] found that adenovirus type 5 was inactivated by the alkaline glycine-NaOH used for elution of adsorbed viruses.

Another deficiency of the evaluation of the TSM is that the method has not been evaluated over a sufficient time period with the same viruses and water source to determine whether changes in water quality influence its effectiveness. Dissolved and colloidal substances in water may interfere with virus recovery, and the types and concentrations of such interfering substances may vary over time.[16,19-21]

In the present study the TSM was rigorously evaluated using all four recommended primary adsorbent filters.[17] Laboratory suspensions of representative viruses from all four major enteric virus groups as well as naturally occurring viruses isolated from sewage were used. Experiments were conducted over 24 months, so that any seasonal variability in TSM efficiency could be detected. Studies were also done to investigate possible virus-specific differences that may influence TSM efficiency. Finally, possible deficiencies inherent in the TSM methodology were examined.

Materials and Methods

Viruses, cell cultures, and virus assays. The nine laboratory viruses used in this study were poliovirus type 1, strain LSc (P-1); coxsackievirus types A9 (CA-9) and B3 (CB-3); echovirus type 7 (E-7); bovine enterovirus type 1 (BE-1); reovirus type 3, Dearing strain (R-3); adenovirus type 5 (Ad-5); simian adenovirus SV-11 (SV-11); and minute virus of mice (MVM). Laboratory viruses were grown and assayed in the following cell cultures: P-1, CB-3, and E-7 in Buffalo Green Monkey kidney (BGMK) cells; CA-9 in RD cells;† R-3, Ad-5, and SV-11 in VERO cells; BE-1 in secondary or tertiary bovine embryonic kidney cells; and MVM in the A9 strain of L cells. In addition, naturally occurring virus mixtures concentrated and purified from raw sewage were used; they were assayed in BGMK cells. All cell cultures were grown and maintained in modified Eagle's minimum essential medium (MEM) with the previously described supplements[22] except the RD cells, which were grown in Eagle's MEM additionally supplemented with nonessential amino acids.

Except for MVM, monodispersed, purified stocks of laboratory viruses were prepared as previously described[22,23] and diluted to approximately 100 plaque-forming units (PFU) per mL in 0.1 M phosphate-buffered saline (PBS) for storage. MVM stocks were prepared by infecting nonconfluent (6×10^4 cells/cm^2 of culture vessel surface area) cultures of A9 L cells at low multiplicity of infection in order to obtain adequate infective virus yields. When cell monolayers showed 75–100 percent cytopathic effects (CPE), they were frozen and thawed three times to maximally disrupt cell membranes. Infected cell debris was recovered by centrifugation at 10 000 × g for 30 min, and pellets were extracted twice with 50 mM tris(hydroxymethyl)-aminomethane buffer containing 1.0 mM disodium ethylene-diamine tetraacetic acid (tris-EDTA) at pH 8.7. Tris-EDTA extracts were then extracted with freon, as previously described for other enteric viruses.[23] Freon-extracted MVM preparations were not subjected to sucrose density gradient centrifugation because electron microscopy revealed that they were monodispersed by the freon extraction.

Purified, dispersed preparations of naturally occurring enteric virus mixtures were obtained by centrifuging 1-L volumes of composite raw sewage at 5000 × g for 1 hr, decanting the supernatant, resuspending the sediment in 20 mL of PBS, and extracting for 2 min with one-half volume of freon. The mixture was centrifuged at 2000 × g for 15 min to break the emulsion, and the aqueous supernatant was combined with the first supernatant. The combined supernatant from freon extraction was centrifuged at 4000 × g for 1 hr to remove large particles including large virus aggregates, and the resulting supernatant was collected, treated with antibiotics (400, 170, and 6.3

†Number 136, American Type Culture Collection, Rockville, Md.

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μg/mL of kanamycin, gentamycin, and fungizone, respectively) and saved as purified, dispersed natural virus stock. All dispersed preparations of laboratory and field viruses were stored at either 4°C or −70°C until used.

Viruses were assayed using the previously described modifications of the plaque technique.[22] Virus stock titers were verified for each experiment by replicate inoculation of three to five cell culture flasks. Samples containing relatively low virus levels were assayed by inoculating a 1 or 3-mL volume into 25 or 75-cm^2 cell culture flasks, respectively, and then allowing 1 to 2 hr for virus adsorption. All enteroviruses were assayed by the procedures previously described for poliovirus.[22] Reovirus was assayed by procedures identical to those for enteroviruses, except that 0.017 percent pancreatin was added to the agar overlay medium.

MVM was assayed by inoculating drained, 1 to 6-hr-old A9 L cell cultures containing 6×10^4 cells/cm^2 of flask surface area. After 1 to 2 hr at 37°C for virus adsorption, the cultures were overlaid with a medium consisting of MEM, 7.5 percent heat-inactivated fetal calf serum (FCS), 1.0 percent agar, 0.4 percent $NaHCO_3$, 0.017 percent pancreatin, and double strength antibiotics. After incubating for five to seven days at 37°C, a second agar overlay containing 0.0015 percent neutral red was applied. The cultures were reincubated at 37°C, and plaques were counted over the next several days.

SV-11 and Ad-5 were assayed on confluent monolayers of VERO cells by procedures similar to those for MVM. Prior to inoculation of Ad-5, cell monolayers were pretreated for 24 hr with 0.05 mg/mL iodo-deoxyuridine.[24] Agar overlay media were supplemented with 2.0 percent heat-inactivated FCS and 0.1 mg/mL diethylaminoethyl dextran instead of pancreatin.

In order to determine the spectrum of naturally occurring virus types isolated from sewage, randomly selected viruses were isolated from plaques on BGMK cell monolayers. Plaque fluid was withdrawn from beneath the overlay agar and inoculated into fresh BGMK cell monolayers in 29-mL (1-oz) glass prescription bottles. After two passages on BGMK monolayers, virus lysates were titered and identified using Lim-Benyesh-Melnick enterovirus antiserum pools[25] on BGMK cells grown in plastic microtiter dishes.

Virus concentration procedures. *Single-step experiments.* In virus concentration experiments involving tap water volumes of 12 L or less, 47-mm diameter, 5 and 1-μm porosity fiberglass-asbestos-epoxy filter series* were used. In these experiments, volumes of cold Chapel Hill (N.C.) tap water were dechlorinated with 10 mg/L of sodium thiosulfate. Aliquots were adjusted to desired pH levels with 1.0 or 0.1 N HCl or NaOH.

Tap water aliquots were seeded with a small volume of monodispersed stock virus to give a desired virus input. Depending on their volumes, the aliquots were placed in either 600-mL capacity polycarbonate or 20-L capacity stainless steel pressure vessels. If the input virus levels were high, small samples were taken for virus assay. The seeded tap water aliquots were filtered through microporous filters using a regulated nitrogen gas cylinder as a source of positive pressure. When the virus inputs were high, the filtrates were collected, sampled, and assayed for viruses. Adsorbed viruses were recovered from the filters by passing two successive 7.5-mL volumes of 0.05 M glycine-NaOH at pH 11.5 through the filters and collecting the eluates dropwise. The pH of the combined eluate was measured and adjusted to pH 7.4 with 0.05 M glycine-HCl at pH 1.5. After measuring the total volume, the eluate was assayed for viruses.

Two-step experiments. Viruses were concentrated from 380-L (100-gal) volumes of seeded Chapel Hill tap water by the two-step procedures described in *Standard Methods*.[17] In these experiments monodispersed viruses were continuously added to the flowing tap water at approximately 1 PFU/gal via one pump of a triplex fluid proportioner* along with the sodium thiosulfate solution used for dechlorination. The volume of thiosulfate-virus feed solution drawn by each fluid proportioner was determined at the end of each experiment and used to calculate virus input. To acidify the tap water to pH 3.5 prior to virus adsorption, HCl was added to the flowing tap water using two alternating pumps of the fluid proportioner. In some experiments the four alternative primary virus adsor-

*Series AA, type M-780, Cox Instrument Div., Lynch Corp., Detroit, Mich.

*Johanson and Son Machine Corp., Clifton, N.J.

TABLE 1

Quality of Raw and Finished Chapel Hill (N.C.) Drinking Water

Year	Months	Turbidity NTU		Color cobalt units		pH		Total Alkalinity mg/L as $CaCO_3$		Total Hardness mg/L as $CaCO_3$		Total Iron mg/L		Total Manganese mg/L		Total Phosphate mg/L		Total Coliforms* CFU/100mL	
		Raw	Finished	Raw	Finished	Raw	Finished	Raw	Finished	Raw	Finished	Raw	Finished	Raw	Finished	Raw	Finished	Raw	Finished
1976	6-7	12.9	0.13	23.0	0.02	7.4	7.1	25.2	21.5	29.4	35.2	0.22	0.02	0.10	T†	0.92	1.76	15.5	0
	8-9	12.4	0.08	24.2	0	7.1	7.1	28.2	28.2	31.6	33.4	0.28	0.02	0.12	0.05	0.11	1.44	10.0	0
	10-11	8.7	0.07	40.7	1.40	7.2	7.0	35.7	34.7	31.6	32.2	0.43	0.07	0.30	0.04	ND‡	1.65	9.0	0
	12-1	17.8	0.10	42.0	0	7.0	7.2	26.0	28.5	32.0	32.0	0.35	0.04	0.18	0.02	1.14	ND	12.4	0
1977	2-3	26.4	0.12	55.0	0	7.0	7.2	19.0	19.0	26.0	26.5	0.42	0.06	0.15	0.02	ND	1.71	23.6	0
	4-5	13.7	0.09	35.7	0	7.0	7.1	21.2	22.0	22.4	22.4	0.36	0.04	0.19	0.02	ND	1.86	19.8	0
	6-7	10.8	0.08	59.0	0	7.1	7.1	29.5	28.5	25.0	28.5	0.74	0.02	0.26	0.01	ND	0.63	14.6	0
	8-9	9.1	0.11	80.5	0	6.9	7.2	28.5	30.5	26.5	28.0	0.26	0.04	0.24	0.03	ND	1.68	38.6	0
	10-11	18.4	0.12	136.0	0.04	7.0	7.3	27.0	30.5	27.0	26.5	0.55	0.04	0.38	0.04	1.80	ND	40.3	0
	12-1	28.9	0.22	185.5	0.20	6.8	7.3	20.5	24.0	27.0	27.0	0.50	0.04	0.02	0.01	ND	1.34	48.5	0
1978	2-3	45.5	0.24	97.5	0.20	6.4	7.2	15.0	19.0	23.5	24.0	0.66	0.05	T	0	0.65	ND	25.5	0
	4-5	32.0	0.16	24.5	0.20	6.4	7.2	16.5	17.0	21.0	21.0	0.62	0.04	T	T	0.53	0.49	49.5	0
	6-7	18.5	0.11	36.0	0.06	6.6	7.2	23.0	30.5	26.5	26.5	0.51	0.04	0.08	T	0.47	0.49	18.0	0
	8-9	21.5	0.26	18.5	0.30	6.7	7.5	28.5	36.0	24.5	24.0	0.35	0.04	0.15	T	ND	0.55	27.0	0
	10-11	23.0	0.16	12.5	0.42	6.6	7.5	32.0	39.5	29.5	29.0	0.58	0.05	0.49	T	ND	0.73	11.0	0
	12-1	22.0	0.28	16.0	1.05	6.8	7.5	31.0	38.0	31.0	32.0	0.42	0.05	0.05	T	ND	ND	5.2	0
	Mean	20.1	0.14	55.8	0.24	6.9	7.2	25.4	27.8	27.2	27.9	0.45	0.04	0.17	0.02	0.80	1.19	23.0	0
	Minimum	8.7	0.07	12.5	0	6.4	7.0	15.0	17.0	21.0	21.0	0.22	0.02	T	0	0.11	0.49	5.2	0
	Maximum	45.5	0.28	185.5	1.40	7.4	7.5	35.7	39.5	32.0	35.2	0.74	0.07	0.49	0.05	1.80	1.86	49.5	0

*Coliforms assayed by MF method; colony-forming units
†Trace
‡Not done

bent systems of the TSM were simultaneously tested with the same tap water and virus type at flow rates of about 0.16 L/s (2.5 gpm). The primary adsorbent filters used were filter 1—a 293-mm diameter, 8 and 1.2-μm porosity mixed cellulose ester filter series;* filter 2—a 2.5-cm (1-in.) diameter by 17.5-cm (7-in.) long, 8-μm porosity, fiberglass-epoxy tube filter;† filter 3—a 267-mm diameter, 5 and 1-μm porosity, fiberglass-asbestos-epoxy filter;‡ and filter 4—a 6.25-cm (2.5-in.) diameter by 25-cm (10-in.) long, 3-μm porosity, fiberglass yarn, honeycomb cartridge filter§ followed by a 127-mm diameter filter 3 series.

In other two-step TSM experiments, four replicate primary virus adsorbent systems (filter 3) were used to simultaneously test four or five different virus types under the same water quality conditions. When five virus types were simultaneously tested with four replicates of a single type of adsorbent, two different virus types having mutually exclusive host cell requirements for plaque assay were mixed together in the sodium thiosulfate feed solution for one adsorbent. After processing, portions of the final concentrate were separately assayed for each virus.

Viruses were eluted from the primary adsorbents with 1 L of 0.05 M glycine–NaOH at pH 11.5, and the collected eluates were adjusted to approximately neutral pH with 0.05 M glycine–HCl at pH 1.5. Viruses in these primary eluates were reconcentrated by adsorption–elution with 47-mm diameter, 5 and 1-μm porosity filter series* using procedures similar to those for the single-stage concentration procedure described above.

Water. Finished Chapel Hill (N.C.) tap water was obtained from a four-place manifold connected to a 2.5-cm (1-in.) diameter water line in the laboratory. Each position on the manifold served a separate fluid proportioner. The raw water source for Chapel Hill tap water is University Lake, a 80-ha (200-acre) impoundment on Morgan Creek that was constructed in 1932. When full, University Lake has a maximum depth of 9 m (30 ft) and a mean depth of 3 m (9.25 ft). As is typical for many small southeastern lakes located in highly vegetated river basins, University Lake is nutrient-rich and contains considerable humic material. The lake is monomictic with a thermocline and clinograde developing during the summer. Stratification persists into the late fall with turnover usually occurring in November. Circulation and mixing continue through the winter and spring, producing high levels of dissolved oxygen. During the summer months of 1976 and 1977 severe drought conditions caused the lake level to drop precipitously, resulting in degradation of water quality. During this period supplemental finished drinking water was obtained from the city of Durham, which is also served by a small surface impoundment on the Flat River called Lake Michie. The Chapel Hill water treatment plant has a multilevel intake structure that allows raw water to be drawn from the depths having the best water quality. During the summer, water is usually drawn from the euphotic zone, thereby avoiding the poorer quality, reduced water in the hypolimnion. In both Chapel Hill and Durham the raw water is treated by a conventional scheme consisting of alum coagulation–flocculation, sedimentation, mixed media (anthracite–sand) filtration, and chlorination. In the warmer summer months potassium permanganate is sometimes added at the coagulation step for taste and odor control. The storage times of the finished water at the Chapel Hill and Durham plants are generally about 1 and 6 hr, respectively. In the Chapel Hill distribution system, water towers may provide as much as one day of storage, depending upon demand. Water quality data for raw and finished Chapel Hill water are given in Table 1.

Results and Discussion

Evaluation of the TSM with 380-L (100-gal) tap water volumes and low enterovirus levels. A series of consecutive experiments was conducted in which the two-stage TSM was evaluated using 380-L (100-gal) tap water volumes, relatively low input levels of single enterovirus types, and the simultaneous parallel operation of the four alternative primary virus adsorbent systems. The mean virus recovery efficiencies from the consecutive evaluation of the five different enteroviruses over a fifteen-month period are given in Table 2 for each of the four primary virus adsorbent systems, along with the mean percent recovery for all four systems.

The recovery of enteroviruses by the two-step TSM was generally poor, ranging from 0.7 to 32 percent, and aver-

*Millipore Corp., Bedford, Mass.
†Grade C, Balston, Inc., Lexington, Mass.
‡Type M-780, series AA, Cox Instrument Div., Lynch Corp., Detroit, Mich.
§Model K27R10S, Commercial Filters Div., Carborundum Co., Lebanon, Ind.

*Series AA, type M-780, Cox Instrument Div., Lynch Corp., Detroit, Mich.

TABLE 2
Enterovirus Recoveries From 380-L (100-gal) Volumes of Tap Water by the Two-Step Tentative Standard Method in Consecutive Experiments

Virus Type	Dates	Number of Replicate Trials	Filter 1*				Filter 2*				Filter 3*				Filter 4*				Overall Mean Recovery§ percent
			Mean Volume		Mean Virus Input† PFU	Mean Recovery‡ percent	Mean Volume		Mean Virus Input PFU†	Mean Recovery‡ percent	Mean Volume		Mean Virus Input† PFU†	Mean Recovery‡ percent	Mean Volume		Mean Virus Input† PFU	Mean Recovery‡ percent	
			L	gal			L	gal			L	gal			L	gal			
P-1	6/24/76 to 9/23/76	13	380	100	69	25 (± 16)	380	100	69	15 (± 7.1)	384	101	70	20 (± 13)	372	98	68	20 (± 17)	22 (± 6.3)
CB-3	10/13/76 to 11/3/76	7	380	100	56	0.7 (± 1.2)	380	100	57	2.6 (± 2.2)	380	100	56	3.7 (± 4.2)	380	100	56	2.8 (± 1.6)	2.4 (± 1.1)
E-7	1/28/77 to 3/8/77	10	274	72	77	10 (± 6.4)	376	99	111	2.5 (± 2.5)	380	100	112	5.2 (± 3.2)	338	89	99	9.2 (± 4.5)	6.7 (± 2.1)
CA-9	4/15/77 to 6/3/77	8	380	100	91	27 (± 16)	380	100	91	16 (± 9.8)	384	101	91	27 (± 12)	372	98	89	32 (± 14)	25 (± 5.9)
BE-1	6/30/77 to 9/20/77	8	372	98	104	18 (± 13)	384	101	110	16 (± 10)	384	101	108	25 (± 22)	380	100	107	28 (± 28)	21 (± 7.8)

*Primary adsorbent type (see text for complete description); secondary absorbent—47-mm diameter, 5 and 1-μm porosity Cox filter series
†Mean calculated total virus input to adsorbent (PFU)
‡Mean percentage of virus input recovered in final concentrate (95 percent confidence interval)
§Mean percentage of virus input recovered with all primary adsorbent types over all trials (95 percent confidence interval); overall mean recovery for all primary adsorbents and all enterovirus types was 16 percent

aging 16 percent. For P-1, C-B3, C-A9, and BE-1, analysis of variance showed no significant differences at the 10 percent level among virus recovery efficiencies of the four alternative primary adsorbents ($p > 0.2$ in all cases), although recovery by filter 2 was generally less efficient than by the other adsorbents. However, analysis of variance for E-7 showed that recovery efficiencies by the various primary adsorbents differed significantly at the 5 percent level ($p = 0.032$).

Further analysis indicated that variable recovery efficiency for E-7 was apparently related to clogging susceptibility of the primary adsorbents. It was observed that filter clogging, as evidenced by process volumes of less than 380 L (100 gal), was most prevalent during the period in which E-7 experiments were performed (January to March 1977). Furthermore, it was noted that filters 1 and 4 frequently clogged (14 percent of all enterovirus trials), while filters 2 and 3 never did. Recovery data for E-7 were grouped and analyzed according to filter clogging. For trials in which filters 1 and 4 processed less than 380 L (100 gal) due to premature clogging, analysis of variance showed that recovery of E-7 varied significantly among the four primary adsorbents ($p = 0.012$). In contrast, for trials in which all adsorbents processed 380 L (100 gal), no significant difference at the 10 percent level (by analysis of variance) was seen among E-7 recovery efficiencies by the different primary adsorbents ($p = 0.556$). Data from trials in which premature clogging occurred were then grouped according to filter type; i.e., filters 1 and 4 (which clogged prematurely) versus filters 2 and 3 (which did not clog). Analysis by paired *t*-test showed that filters 1 and 4 yielded significantly better recoveries at the 5 percent level than did filters 2 and 3 ($p = 0.0008$).

The reasons for increased recovery efficiency of E-7 with clogged filters are not clear and deserve further study. It is possible that premature clogging with particulate material reduced accumulation of soluble-colloidal adsorption interferences on the filter surface, permitting better adsorption of viruses. Alternatively, accumulated clogging materials may themselves have provided additional virus adsorption sites. Finally, it is possible that the reduced flux through a clogged filter improved virus-filter surface interactions to allow increased virus adsorption.

The results in Table 2 indicate that there are appreciable differences in recovery efficiency among the different enteroviruses, regardless of the type of primary virus adsorbent filter system, and this was confirmed at the 5 percent level by analysis of variance ($p < 0.03$ for each adsorbent). Considering that all five virus types belong to the same taxonomic group (enterovirus), the substantial differences in virus recovery efficiency were unexpected, and suggested the disturbing possibility that there might be wide variations in recovery efficiency for the more than seventy types of human enteroviruses.

However, when enterovirus recovery data were plotted as a bimonthly mean histogram (Fig. 1), an apparent temporal variability in virus recovery efficiency was observed. Analysis of variance established that there was significant variation, at the 5 percent level, among bimonthly mean enterovirus recoveries ($p = 2.5 \times 10^{-12}$). Thus the possibility was suggested that water quality changes may affect the TSM and account for observed variations in virus recovery efficiency.

In order to determine whether the observed differences in enterovirus recoveries were caused by real differences in the viruses or by other factors, a series of experiments was performed during the third and final year of the study in which all five enteroviruses were simultaneously tested with a single type of primary adsorbent system; namely, the filter 3 series. Two-step, 380-L (100-gal) experiments were done using TSM adsorption and elution conditions. As in the previous consecutive experiments, the overall mean recovery efficiency for all five virus types was 16 percent (Table 3). Although the results of the experiments show differences in mean recovery efficiencies for the five different enteroviruses, these differences were not significant at the 10 percent level by analysis of variance ($p = 0.111$). Thus the previously observed variations in enterovirus recovery efficiencies with the TSM are apparently due to factors other than actual virus differences.

Evaluation of the TSM with 380-L (100-gal) volumes of tap water and low levels of other enteric viruses. The results of a series of consecutive TSM experiments conducted over a nine-month period with the other four laboratory enteric viruses are summarized in Table 4. For MVM, a parvovirus, the recovery efficiencies by the four alternative primary virus adsorbents were not significant at the 10 percent level by analysis of variance ($p = 0.85$). More important is the fact

TABLE 3

*Enterovirus Recoveries From 380-L (100-gal) Volumes of Tap Water by the Two-Step Tentative Standard Method When Testing Viruses Simultaneously**

Virus Type	Number of Replicate Trials†	Mean Volume		Mean Virus Input PFU	Mean Recovery‡ percent
		L	gal		
P-1	9	391	103	132	7.5 (± 9.0)
CA-9	4	376	99	127	33 (± 30)
CB-3	14	410	108	283	15 (± 10)
E-7	14	410	108	192	23 (± 18)
BE-1	14	403	106	167	9.1 (± 5.4)

*Primary virus adsorbent—267-mm diameter, 5 and 1-μm pore size, filter 3 series.
†Number of trials varies among viruses due to occasional assay failure or virus stock problems resulting in undetectable virus input.
‡Mean percentage virus recovery (95 percent confidence interval); overall mean recovery was 16 percent.

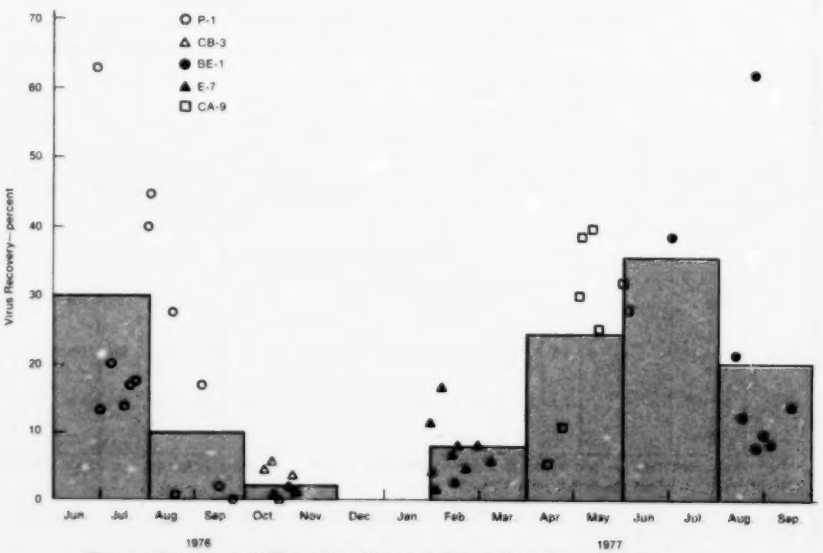

Fig. 1. Histogram of Bimonthly Average Enterovirus Recovery Efficiencies

that the two adenoviruses and reovirus type 3 were not recovered at all. MVM was recovered with an average efficiency of only 5.0 percent, which is significantly lower at the 5 percent level by two-sample *t*-test ($p = 0.0007$) than the mean poliovirus recovery of 22 percent in enterovirus consecutive experiments. Thus enteroviruses, including poliovirus, may not be good models for adeno-, reo-, and parvoviruses with respect to TSM recovery efficiency.

During the third year of the project a series of simultaneous, two-step, 380-L (100-gal) TSM experiments was conducted with the four laboratory enteric viruses to determine if the results from the previous consecutive evaluations

TABLE 4

Enteric Virus Recoveries From 380-L (100-gal) Volumes of Tap Water by the Two-Step Tentative Standard Method in Consecutive Experiments

Virus Type	Dates	Number of Replicate Trials	Filter 1*				Filter 2*				Filter 3*				Filter 4*			
			Mean Volume		Mean Virus Input†	Mean Recovery‡	Mean Volume		Mean Virus Input†	Mean Recovery‡	Mean Volume		Mean Virus Input†	Mean Recovery‡	Mean Volume		Mean Virus Input†	Mean Recovery‡	Overall Mean Recovery§
			L	*gal*	*PFU*	*percent*	*L*	*gal*	*PFU*	*percent*	*L*	*gal*	*PFU*	*percent*	*L*	*gal*	*PFU*	*percent*	*percent*
MVM	10/11/77 to 4/20/78	6	361	95	81	5.1 (± 11)	380	100	74	6.0 (± 13)	388	102	74	1.7 (± 3.1)	380	100	86	7.0 (± 15)	5.0 (± 4.4)
SV-11	11/16/77 to 1/6/78	6	369	97	119	0	372	98	115	0	380	100	120	0	369	97	118	0	0
Ad-5	1/18/78 to 3/10/78	4	353	93	101	0	380	100	116	0	380	100	73	0	315	83	113	0	0
R-3	4/4/78 to 6/19/78	6	384	101	107	0	380	100	109	0	380	100	102	0	380	100	109	0	0

*Primary adsorbent type (see text for complete description); secondary adsorbent—47-mm diameter, 5 and 1-μm porosity Cox filter series
†Mean calculated total virus input to adsorbent (PFU)
‡Mean percentage of virus input recovered in final concentrate (95 percent confidence interval)
§Mean percentage of virus input recovered with all primary adsorbent types over all trials (95 percent confidence interval)

TABLE 5

*Enteric Virus Recoveries From 380-L (100-gal) Volumes of Tap Water by the Two-Step Tentative Standard Method When Testing Viruses Simultaneously**

Virus Type	Number of Replicate Trials†	Mean Volume		Mean Virus Input *PFU*	Mean Recovery‡ *percent*
		L	*gal*		
P-1	10	410	108	77	11 (± 14)
Reovirus type 3	6	422	111	326	0.1 (± 0.2)
SV-11	10	395	104	282	0
Ad-5	11	399	105	201	0
P-1	9	399	105	126	11 (± 16)
MVM	11	395	104	372	3.1 (± 4.0)

*Primary virus adsorbent—267-mm diameter, 5 and 1-μm pore size, filter 3 series
†Number of trials varies among viruses due to occasional assay failure or virus stock problems resulting in undetectable virus input.
‡Mean percentage virus recovery (95 percent confidence interval)

TABLE 6

Recovery of Natural Enteric Virus Mixtures From 380-L (100-gal) Volumes of Experimentally Seeded Tap Water by the Tentative Standard Method

Date	Trial Number	Filter 1		Filter 2		Filter 3		Filter 4		All Filters
		Virus Input *PFU*	Virus Recovery *percent*	Virus Input *PFU*	Virus Recovery *percent*	Virus Input *PFU*	Virus Recovery *percent*	Virus Input *PFU*	Virus Recovery *percent*	Virus Recovery *percent*
12/19/77	1	50	< 2.2	42	< 2.7	32	3.6	41	< 2.7	0.9
01/30/78	2	22	5.2	21	< 5.4	20	< 5.5	9.5	11.8	4.3
02/01/78	3	14	8.1	25	< 4.6	25	< 4.5	5.9	< 19	2.0
02/15/78	4	36	3.1	40	2.8	41	< 2.7	14	< 8.1	1.5
04/26/78	5	15	7.3	22	15.5	21	< 5.2	22	4.8	8.9
04/28/78	6	23	9.8	33	3.7	32	< 3.2	32	< 3.2	3.4
06/13/78	7	40	< 3.4	42	2.8	41	26	38	< 2.8	7.3
06/27/78	8	16	< 6.3	18	5.9	19	6.7	18	< 5.8	3.2
07/14/78	9	13	18	14	< 8.3	14	8.1	12	9.0	8.8
07/26/78	10	13	< 7.9	14	< 8.1	12	< 9.2	14	< 8.7	0
08/07/78	11	70	1.6	68	3.3	64	7.4	61	7.4	4.9
08/21/78	12	39	< 3.3	41	< 3.1	39	5.8	37	9.5	3.8
09/07/78	13	33	< 3.3	36	< 3.1	38	< 2.9	81	< 3.5	0
09/29/78	14	87	1.2	92	6.2	86	1.5	83	8.1	4.3
10/09/78	15	42	6.0	42	1.6	41	5.3	40	14	6.4
10/23/78	16	143	< 0.7	145	< 0.7	142	0.9	129	1.0	0.5
11/10/78	17	38	12	37	< 2.9	40	2.7	35	16	7.8
	Mean*	41	4.3	43	2.5	42	4.0	37	4.8	3.9

*Less-than values assigned values of 0 percent for calculation of means

with these viruses could be confirmed. The primary virus adsorbent in these experiments was again the filter 3 series, and poliovirus was simultaneously evaluated to act as a control. Experiments were conducted in two sets: those in which SV-11, Ad-5, R-3, and P-1 were concentrated simultaneously, and those in which MVM and P-1 were concentrated simultaneously. As in the previous experiments SV-11 and Ad-5 were not recovered at all, while R-3 was just barely detected (Table 5). MVM recovery efficiency (3.1 percent) was somewhat lower than in the previous experiments (5.0 percent), but was not significantly different at the 10 percent level by two-sample *t*-test ($p = 0.253$) than the recovery efficiency for poliovirus (11 percent). The poliovirus recovery efficiency in either set of experiments (11 percent, Table 5) was not significantly different at the 10 percent level by two-sample *t*-test ($p = 0.337$) from that in the simultaneous enterovirus experiments (7.5 percent, Table 3). The results of these experiments indicate that the ineffectiveness of the TSM for concentrating the representative adeno- and reoviruses is indeed due to virus-specific factors. In the case of MVM, poor recovery efficiencies observed in consecutive experiments (Table 4) may have been due to temporal variations in water quality, as with the enteroviruses.

Evaluation of the TSM with 380-L (100-gal) volumes of tap water and low levels of natural virus mixtures obtained from raw sewage. The results of a series of 380-L (100-gal) two-step TSM experiments done over a twelve-month period with tap water seeded with natural virus mixtures obtained from raw sewage are summarized in Table 6. Virus recoveries were generally low, ranging from < 1 to 26 percent and averaging 3.9 percent. In similar experiments with poliovirus type 1 performed during the same time period but on different days, virus recoveries ranged from < 1 to 47 percent and averaged 4.1 percent.

Recoveries of poliovirus and natural virus mixtures during this time period were not significantly different at the 10 percent level by two-sample *t*-test ($p = 0.902$). This result is not surprising, since all viruses isolated by plaque purification from natural virus mixtures were enteroviruses (Table 7).

Investigation of possible causes of poor virus recovery by the TSM. *Effects of tap water quality on enteric virus recovery.* The observation that enterovirus recoveries appeared to vary over time in consecutive experiments (Fig. 1) and yet were not significantly different when tested simultaneously suggested that the variations in the consecutive experiments may be related to temporal changes in water quality. Indeed, previous studies have shown that filter adsorption–elution methods for virus concentration are sensitive to differences in water quality, and that both soluble (or colloidal) and particulate constituents can interfere with virus recovery.[13,16,19,20,26-30] The present study was done with finished tap water having an average turbidity of 0.1 NTU (Table 1); therefore, it is unlikely that particulate matter was responsible for the observed differences in enterovirus recovery efficiency. However, because previous studies have shown that soluble or colloidal organic matter can reduce virus recovery by interfering with virus adsorption to filters,[13,16,20,26,29] experiments were performed to determine whether the poor virus recoveries of this study were related to such interferences.

If soluble or colloidal materials in tap water accumulate on adsorbents and interfere with virus adsorption, the virus adsorption efficiency should increase with decreasing tap water process volume. Improved adsorption should be reflected as improved recovery when identical elution conditions are used. A series of single-step concentration experiments was conducted in which equal numbers (20–1300 PFU) of poliovirus or MVM were concentrated from 500-mL and 12-L volumes of dechlorinated tap water using parallel 47-mm diameter filter series. The adsorption and elution conditions were as specified in the TSM. The recovery efficiencies of the viruses from the two volumes of tap water are shown in Table 8. For both poliovirus and MVM, recovery was two to three times more efficient when viruses were concentrated from 500 mL than when concentrated from 12 L. Recovery differences for each virus were significantly different at the 5 percent level by paired *t*-test (poliovirus—$p = 0.003$; MVM—$p = 0.002$). The results of these experiments support the hypothesis of water quality influences on TSM recovery efficiency, because the larger tap water volume produced increased interference with virus adsorption to filters, presumably because of its greater quantity of soluble or colloidal materials.

Investigations were then conducted to determine whether removal of organic materials from tap water could improve virus adsorption and recovery. In the initial experiments samples of tap water were slowly filtered through granular activated carbon (GAC) to remove organics. Samples of both GAC-treated and untreated tap water were adjusted to pH 3.5 for virus adsorption and seeded with about 5×10^3 PFU per mL of selected viruses. Twelve or 6-L volumes were then filtered through the 47-mm diameter filter series, and the filtrates were sampled and assayed for viruses. As shown by the results in Table 9, GAC treatment of the tap water resulted in considerable improvement in virus adsorption compared to untreated control tap water. It should be noted, however, that GAC treatment of water for removal of interfering substances prior to virus adsorption cannot be used in a concentration method for indigenous viruses because the viruses might also be removed and hence never reach the adsorbent filter.

To further evaluate the effect of GAC treatment on virus recovery efficiency, single-step concentration experiments were done using 12-L volumes of GAC-

TABLE 7
Identification of Viruses Isolated From Natural Virus Mixtures and From TSM Concentrates of Natural Viruses

Sample Date	Origin of Isolate	Virus Type	Number of Isolates
6/13/78	sewage extract	P-1	2
	sewage extract	poliovirus type 2	3
	sewage extract	poliovirus type 3	1
6/13/78	TSM concentrate	poliovirus type 2	2
8/7/78	sewage extract	E-7	1
8/7/78	TSM concentrate	poliovirus type 2	1
8/21/78	sewage extract	E-7	2
	sewage extract	enterovirus*	1

*Isolate neutralized by all Lim–Benyesh-Melnick enterovirus antiserum pools

TABLE 8
*Effect of Tap Water Volume Processed on Virus Recovery Efficiency**

Virus Type	Experiment Number	Virus Input PFU	Virus Recovery—percent	
			0.5 L	12 L
P-1	1	22	45	39
	2	71	37	3.2
	3	39	38	54
	4	454	56	18
	5	264	21	< 1.0
	6	305	50	18
	7	106	28	9.6
	8	535	54	4.0
	9	116	99	48
	mean		48	22
MVM	1	1298	54	4
	2	672	> 99	48
	3	1338	90	28
	mean		81	27

*Virus adsorbent—47-mm diameter, 5 and 1-μm pore size Cox filter series; elution with 15 mL of pH 11.5 glycine–NaOH

TABLE 9
*Effect of Tap Water Treatment With Granular Activated Carbon on Virus Adsorption to Filter 3 Series**

Virus Type	Virus Adsorption Efficiency percent	
	Untreated Tap Water	Carbon-Filtered Tap Water
P-1	40	58
SV-11	22	86
MVM	68	84
Mean, all viruses	43	76

*Tap water volumes—12 L for P-1 and MVM, 6 L for SV-11; adsorption pH—3.5; filters—47-mm diameter, 5 and 1-μm porosity Cox filter series; initial virus concentration in tap water—≈ 5×10^3 PFU/mL

treated and untreated tap water with initial virus levels of about 100 to 1000 PFU, adsorption at pH 3.5, and elution with pH 11.5 glycine–NaOH. As shown by the results in Table 10, BE-1 and MVM recoveries were significantly better at the 5 percent level by paired *t*-test for the GAC-treated water than for the untreated control (BE-1–$p = 0.006$; MVM–$p = 0.003$). Although the results of these experiments suggest that soluble or colloidal organic matter or some other tap water constituents removed by GAC appear to interfere with virus adsorption to filters, the results with the other enteroviruses showed that this was not a constant phenomenon. For P-1, CB-3, C-A9, and E-7 the recovery efficiencies with GAC-treated and untreated tap water were not significantly different at the 10 percent level by paired *t*-test ($p > 0.16$ for each virus). Possibly, there were fewer interferences in the tap water removable by GAC during the experiments with P-1, E-7, C-A9, and CB-3, and, therefore, virus adsorption was not significantly improved. Alternatively, the viruses tested may be affected to varying degrees by adsorption interferences in tap water.

Effect of pH on enteric virus stability under TSM conditions. The stability of representative viruses from the four enteric virus groups in tap water and 0.05 *M* glycine–NaOH at the various pH extremes encountered in the TSM was evaluated in an effort to determine whether this factor played some role in the poor virus recoveries that had been obtained. Volumes of tap water and glycine were adjusted to the pH levels shown in Table 11. While vigorously mixing magnetically, a small amount of stock virus was added to give an initial concentration of about 2 to 5 × 10^5 PFU/mL. Samples for virus assay were taken at intervals over a total reaction period of 120 min, and they were neutralized by dilution in PBS. The results of these experiments are summarized in Table 11 in terms of T-90 values; i.e., the time in minutes for 90 percent of the initial viruses to become inactivated. All of the viruses were quite stable in tap water at pH 3.0–3.5 and pH 7.0–7.5, with T-90 values in excess of 120 min. Therefore, it is unlikely that the poor virus recoveries of the TSM can be attributed to adverse effects of tap water pH. However, marked differences in virus stability were observed in glycine at high pH levels. At the TSM elution pH of 11.5, the representative adenovirus, reovirus, and enterovirus were extremely unstable, with T-90 values of less than 1 min for SV-11 and R-3, and 1.8 min for P-1. MVM was also rather labile under these conditions with a T-90 value of 20 min. The results of these experiments indicate that poor virus recoveries by the TSM can be at least partially attributed to virus inactivation during elution with pH 11.5 glycine–NaOH.

Summary and Conclusions

In this study virus recovery efficiencies achieved by the TSM were generally poor. For enteroviruses (P-1, CA-9, CB-3, E-7, and BE-1) the recovery ranged from 0.7 to 32 percent, and averaged 16 percent during fifteen months of evaluation. The representative parvovirus, MVM, was recovered with an average efficiency of only 5 percent; the representative reovirus and adenoviruses were not recovered at all during nine months of evaluation. Average recovery efficiency of natural enterovirus mixtures obtained from raw sewage was 3.9 percent.

Although no statistically significant difference occurred among enteric virus recovery efficiencies by the four alternative primary adsorbents, filter 2 yielded generally lower enterovirus recoveries than did filters 1, 3, and 4. Furthermore, simultaneous evaluations with the five enteroviruses and the filter 3 primary adsorbent showed no differences among recovery efficiencies for these viruses. However, temporal variability in the enterovirus recovery efficiency suggested that water quality changes influenced the effectiveness of the TSM.

The results of the single-step, small-volume concentration experiments verified that tap water constituents can interfere with virus adsorption to filter surfaces. Virus adsorption efficiency was directly dependent on the volume of tap water (i.e., the amount of interfering tap water components) processed through the adsorbent. Moreover, removal of organic materials from tap water with granular activated carbon before filtration improved virus adsorption and significantly improved recovery of at least two enteric virus types.

Representative viruses from all four major enteric virus groups were shown to be unstable in 0.05 *M* glycine–NaOH at pH 11.5, the recommended eluent in the TSM. Ninety percent inactivation of viruses occurred in less than 1 min for R-3 and SV-11, 1.8 min for P-1, and 20 min for MVM. Thus the poor or nonexistent virus recoveries observed in this study can be at least partially attributed to virus inactivation by pH 11.5 glycine–NaOH during elution from filters.

The results of this study indicate that the present TSM has inherent major deficiencies. Modifications of the method will be necessary to circumvent interference with virus adsorption by tap water constituents and inactivation of viruses during elution. The investigation of possible modifications of the TSM is the subject of the authors' next article.

TABLE 10
*Effect of Tap Water Treatment With Granular Activated Carbon on Enteric Virus Recovery**

Virus Type	Number of Replicate Trials†	Mean Virus Input PFU	Virus Recovery‡ percent	
			Untreated Tap Water	GAC-Treated Tap Water
P-1	7	180	20 (± 18)	21 (± 13)
CB-3	5	211	3.1 (± 4.2)	3.8 (± 3.8)
CA-9	3	1198	1.9 (± 1.9)	9.0 (± 16)
E-7	5	265	34 (± 32)	42 (± 43)
BE-1	6	110	17 (± 14)	32 (± 13)
MVM	3	1198	27 (± 55)	52 (± 58)

*Tap water volume–12 L; adsorption pH–3.5; filters–47-mm diameter, 5 and 1-μm porosity Cox filter series; eluent–pH 11.5 glycine–NaOH
†Number varies among virus types due to occasional assay failure or virus stock problems resulting in undetectable input.
‡Mean percentage recovery of virus input (95 percent confidence interval)

TABLE 11
Effect of pH on Enteric Virus Stability in Tap Water and Glycine–NaOH

Virus Type*	Time for 90 Percent Virus Inactivation–min					
	Tap Water pH		Glycine pH			
	3.0–3.5	7.0–7.5	9.5	10.5	11.0	11.5
MVM	> 120	> 120	> 120	> 120	ND†	20
P-1	> 120	> 120	> 120	> 120	38	1.8
Reovirus type 3	> 120	> 120	> 120	24	< 1	< 1
SV-11	> 120	> 120	> 120	2.8	< 1	< 1

*Initial virus concentration–2–5 × 10^5 PFU/mL
†Not done

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References

1. CRAUN, G. & GUNN, R.A. Outbreaks of Waterborne Disease in the United States. *Jour. AWWA*, 71:8:422 (Aug. 1979).
2. TODD, E.C.D. Waterborne Disease in Canada, 1974–1975. Proc. 1978 AWWA Ann. Conf., Atlantic City, N.J. (1978).
3. HOEHN, R.C. ET AL. Trihalomethanes and Viruses in a Water Supply. *Jour. Envir. Engrg. Div.–ASCE*, 103:EE5:803 (Oct. 1977).
4. METCALF, T.G. ET AL. Incidence of Virus in Water. Proc. 1978 AWWA Ann. Conf., Atlantic City, N.J. (1978).
5. COIN, L. ET AL. Modern Microbiological and Virological Aspects of Water Pollution. *Advances in Water Pollution Res., Proc. Second Intern. Conf.* Pergamon

Press, New York (1965).
6. NUPEN, E. Viruses in Renovated Waters. *Viruses in Water* (G. Berg et al, editors). APHA, Washington, D.C. (1976).
7. DROSDOV, S.G. & KASANTSEVA, V.A. Detection of Viruses in Sewage, Surface, and Tap Waters: Effectiveness of Treatment in Removal of Viruses. *Intern. Virology IV, Abstracts of the Fourth Intern. Congr. for Virology*. Centre for Agr. Publ. and Documentation, Wageningen, Netherlands (1978).
8. BERG, G. ET AL. *Indicators of Viruses in Water and Food*. Ann Arbor Science Publ., Ann Arbor, Mich. (1978).
9. GERBA, C.P.; WALLIS, C.; & MELNICK, J.L. Viruses in Water: The Problem, Some Solutions. *Envir. Sci. Technol.*, 9:13:1122 (Dec. 1975).
10. BERG, G. ET AL. *Viruses in Water*. APHA, Washington, D.C. (1976).
11. SOBSEY, M.D. Enteric Viruses and Drinking Water Supplies. *Jour. AWWA*, 67:8:414 (Aug. 1975).
12. SOBSEY, M.D. Detection Methods for Enteric Viruses in Water and Wastewater. *Viruses in Water* (G. Berg et al, editors). APHA, Washington, D.C. (1976).
13. FARRAH, S.R. ET AL. Concentration of Viruses from Large Volumes of Tap Water Using Pleated Membrane Filters. *Appl. Envir. Microbiol.*, 31:2:221 (Feb. 1976).
14. HILL, W.J. JR. ET AL. Detection of Virus in Water: Sensitivity of the Tentative Standard Method for Drinking Water. *Appl. Envir. Microbiol.*, 31:2:254 (Feb. 1976).
15. JAKUBOWSKI, W.; HILL, W.F. JR.; & CLARKE, N.A. Comparative Study of Four Microporous Filters for Concentrating Viruses from Drinking Water. *Appl. Envir. Microbiol.*, 31:1:58 (Jul. 1975).
16. SOBSEY, M.D. ET AL. Concentration of Enteroviruses from Large Volumes of Water. *Appl. Microbiol.*, 26:4:529 (Oct. 1973).
17. *Standard Methods for the Examination of Water and Wastewater*. APHA, AWWA, and WPCF, Washington, D.C. (14th ed., 1976).
18. FIELDS, H.A. Environmental Parameters Influencing the Recovery of Adenovirus from Surface Waters. Doctoral thesis, Univ. of New Hampshire, Durham, N.H. (1974).
19. FARRAH, S.R. ET AL. Characteristics of Humic Acid and Organic Compounds Concentrated from Tap Water Using the Aquella Virus Concentrator. *Water Res.*, 10:10:897 (Oct. 1976).
20. FARRAH, S.R. ET AL. Reconcentration of Poliovirus From Sewage. *Appl. Microbiol.*, 32:5:654 (Nov. 1976).
21. WALLIS, C.; HENDERSON, M.; & MELNICK, J.L. Enterovirus Concentration on Cellulose Membranes. *Appl. Microbiol.*, 23:3:476 (Mar. 1972).
22. SOBSEY, M.D.; JENSEN, H.R.; & CARRICK, R.J. Improved Methods for Detecting Enteric Viruses in Oysters. *Appl. Envir. Microbiol.*, 37:1:121 (Jul. 1978).
23. FLOYD, R. & SHARP, D.G. Aggregation of Poliovirus and Reovirus by Dilution in Water. *Appl. Envir. Microbiol.*, 33:1:159 (Jan. 1977).
24. STAAL, S.P. & ROWE, W.P. Enhancement of Adenovirus Infection in SI-38 and AGMK Cells by Pretreatment of Cells with 5-Iododeoxyuridine. *Virology*, 64:513 (1975).
25. Procedure for Using the Dried Lim/Benyesh-Melnick Pools for Typing Enteroviruses. Natl. Inst. of Allergy and Infectious Diseases, NIH, DHEW, Bethesda, Md. (Apr. 1972).
26. HOMMA, A. ET AL. Virus Concentration From Sewage. *Water Res.* 7:7:945 (Sep. 1973).
27. WELLINGS, F.M.; LEWIS, A.L.; & MOUNTAIN, C.W. Demonstration of Solids-Associated Virus in Wastewater and Sludge. *Appl. Envir. Microbiol.*, 31:3:354 (Mar. 1976).
28. SCHAUB, S.A. & SAGIK, B.P. Association of Enteroviruses with Natural and Artificially Introduced Colloidal Solids in Water and Infectivity of Solids-Associated Virions. *Appl. Microbiol.*, 30:2:212 (Aug. 1975).
29. WALLIS, C. & MELNICK, J.L. Concentration of Viruses from Sewage by Adsorption on Millipore Membranes. *Bull. World Health Org.*, 36:2:219 (Aug. 1967).
30. SOBSEY, M.D. ET AL. Development of Methods for Detecting Viruses in Solid Waste Landfill Leachates. *Appl. Microbiol.*, 28:2:232 (Aug. 1974).

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